APPLICATION FOR UNITED STATES LETTERS PATENT

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TITLE: DEVICE FOR ANNALYZING IMMUNOASSAYS

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CROSS-REFERENCE TO RELATED APPLICATION

This application claims the priority of European Patent Application 99 116 534.1, filed with the European Patent Office on August 24, 1999, the subject matter of which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

The invention relates to a device for analyzing immunoassays with a liquid assay medium which is limited by at least one boundary surface of a solid body, wherein first reaction agents are dissolved in the assay medium and are labeled with a luminophore or different luminophores and second reaction agents are bonded to the boundary surface within a boundary layer of the assay medium, and wherein for a quantitative detection of the first reaction agents with the aid of light rays emitted by a transmitter, an evanescent field is generated through which luminophore-labeled first reaction agents that are bonded to the second reaction agents are optically excited and emit fluorescent rays and/or phosphorescent rays that can be detected in a receiver

The field of medical diagnostics, specifically immunological diagnostics, is based to a high degree on the

ELISA (enzyme-linked immunosorbent assay). A recent overview of immunoassays can be found in Hage, Anal. Chem., Vol. 71, pages 294R - 304R, 1999. Two features characterize an ELISA. A first reaction agent is labeled with an enzyme and is dissolved in an assay medium. A second reaction agent is bonded to a solid phase, wherein the solid phase is formed by a boundary surface of a solid body that limits the assay medium.

Standardized plastic panels, frequently made of polystyrene, which contain 96 wells are primarily used for the solid phase. The plastic well surface bonds proteins, which form the second reaction agents, through adsorption in the nanogram range. This amount is sufficient for an immunological detection. A bonding reaction with the first, enzyme-labeled reaction agent present in the solution leads to the bonding of the enzyme to the solid phase. The bonded enzyme is made visible through adding a chromogen substrate that is specific for this enzyme. Subsequently, the resulting colored product can be evaluated optically.

A number of technical options exist for labeling the first reaction agent, mostly an immunoglobulin, with an

enzyme. Peroxidase or alkaline phosphatase is commonly used for the labeling.

Excellent results can be obtained with ELISAs with respect to sensitivity and specificity. The achievable detection limits are in the nanogram range or below.

Assays based on this principle are realized in the most varied forms and are used for detecting antigens or anti-bodies, depending on the problem definition.

However, one significant disadvantage of the ELISA is the handling of the tests because different reagents must be added successively to the wells and must also be removed again. The total of the various pipetting, washing, and incubation steps is different from assay to assay and can number ten or more. For that reason, ELISAs are time-consuming and their performance is very involved operationally. ELISAs must be carried out with great precision by specially trained personnel to achieve good results.

Another disadvantage of the ELISA is the time required for an assay, which is determined by the sum of the incubation and washing steps and normally lasts from one to several hours.

A device for analyzing immunoassays is known from US 3,939,350, for which a transparent disk can be inserted between a prism and a container holding the assay medium.

By means of a laser, transmitted light rays are beamed at the total reflection angle onto the disk, thereby creating an evanescent field in the border region of the assay medium, in the disk area.

The first reaction agent in the solution is labeled with a luminophore. The second reaction agent is bonded to the surface of the disk. If the luminophore-labeled reaction agent in the solution bonds to the surface of the disk, it can subsequently be excited by the evanescent field of a totally reflected light ray and can emit fluorescent radiation. This fluorescent radiation is determined quantitatively and is directly proportional to the bonded luminophore-labeled reaction agent and is thus directly proportional to the amount of the originally existing reaction agent in the solution.

Since only the luminophore bonded to the surface is positioned in the field of evanescence of the laser beam, only this bonded luminophore is optimally excited and emits photons. A luminophore in the solution that is not bonded is not positioned in the field of evanescence of the light

beam, is therefore not excited and consequently also does not emit photons. This arrangement therefore permits the quantitative determination of bonded luminophore in the presence of non-bonded luminophore.

However, the disadvantage in this case is the mechanically complicated design of the device. In particular the preparation of the disk and the subsequent insertion of the disk between prism and container are extremely time-consuming. In addition, the preparation can only be carried out by qualified personnel.

SUMMARY OF THE INVENTION

It is an object of the invention to provide a device of the aforementioned type such that an analysis of immunoassays can be carried out with high detection sensitivity and a low expenditure in time, cost and material.

The above and other objects are accomplished according to the invention by the provision of a device for analyzing immunoassays with a liquid assay medium, comprising: a vessel for holding the assay medium and having a base comprised of a solid body, the solid body having a first side wall and a top surface constituting a bottom surface

of the vessel and forming a boundary surface of the solid body, wherein first reaction agents are dissolved in the assay medium in the vessel and are labeled with a luminophore or different luminophores and second reaction agents are bonded to the boundary surface within a boundary layer of the assay medium; a transmitter for emitting light rays that are coupled into the base of the vessel via the first side wall and conducted at the total reflection angle to the boundary surface so that luminophore-labeled first reaction agents that are bonded to the second reaction agents are optically excited by at least some of the light rays and emit at least one of fluorescent and phosphorescent rays; and a receiver positioned for quantitatively detecting the at least one of the fluorescent rays and phosphorescent rays.

Thus, according to the invention, the assay medium with the first and second reaction agents as well as the luminophore is located inside a vessel, wherein the second reaction agent is bonded to the top surface that forms the boundary surface of the base of the vessel.

The light rays emitted by the transmitter are coupled in and refracted via a wall on the side of the base, so that these rays travel in the base and are conducted onto

the boundary surface at the angle of total reflection. The fluorescent rays and/or the phosphorescent rays emitted by the luminophores are recorded inside the receiver.

The light-deflecting and light-conducting means for producing the field of evanescence thus can be produced simply and cheaply through a suitable design of the vessel base. It is particularly advantageous in this case that the light-deflecting and light-conducting means, as well as the receptacle for the assay medium, are formed by a vessel that is preferably designed as one piece.

Preferably, the upper edge of the vessel has an additional attachment for fastening the vessel to a holder.

The analysis of immunoassays thus can be carried out with a low number of operational steps. In particular, hardly any effort is required for preparing the assays.

It is also advantageous that the device according to the invention comprises few individual parts, requires little assembly effort and also has small dimensions as well as a low weight, so that it is easy to handle and in particular can also be designed to be mobile.

An especially advantageous embodiment can also provide for an arrangement of multiple vessels, wherein these vessels are stationary arranged and can be examined with

the aid of a multiple arrangement of transmitters. In this case, a common receiver records the fluorescent rays and/or the phosphorescent rays coming from the individual vessels.

This arrangement is simple and can be produced cheaply. Furthermore, it is advantageous that no moving parts are required for this arrangement, thereby keeping the calibration expenditure low.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features and advantages of the invention will be further understood from the following detailed description of the preferred embodiments with reference to the accompanying drawings.

Figure 1 is a schematic representation of a first exemplary embodiment of a device for analyzing immunoassays, comprising a vessel for holding an assay medium.

Figure 2 is a cross section through the vessel according to Figure 1.

Figure 3 is a view from above of the vessel according to Figure 1.

Figure 4 is a partial section of a second embodiment for analyzing immunoassays.

Figure 5 is a schematic illustration of a third embodiment for analyzing immunoassays with a first multiple arrangement of vessels.

Figure 6 is a cross section through a portion of the device according to Figure 5.

Figure 7 is a schematic illustration of a third embodiment for analyzing immunoassays with a second multiple arrangement of vessels.

Figure 8 is a cross section through the device according to Figure 7.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Figure 1 shows a first embodiment of a device 1 for analyzing immunoassays according to the invention. The immunoassay to be analyzed is located in a vessel 2. An optical sensor arrangement is provided for analyzing the assay. The sensor arrangement comprises a transmitter 4 in the form of a laser, which emits light rays 3. A polarization filter 5 is installed downstream of transmitter 4 for a linear polarization of light rays 3, which are focused onto vessel 2.

Transmitter 4 is arranged in front of a slanted sidewall 6 of a base 2' of vessel 2. The slant for side

wall 6 is selected such that the arriving light rays 3 are refracted at side wall 6 toward a top surface 7 of base 2' and arrive there at the angle of total reflection. The inclination of sidewall 6 furthermore is selected such that light rays 3 arrive at the Brewster angle. As a result of this, the polarization of the light rays 3 remains the same when entering vessel 2. In addition, there are no intensity losses. The top surface 7 of base 2' constitutes a boundary surface for the immunoassay. For convenience surface 7 is alternately referred to herein as the top surface of base 2' or the boundary surface for the immunoassay. Consequently, nearly the total amount of light from transmitted light rays 3 is reflected at top surface 7 of the base and conducted to a second side wall 6' of base 2' of vessel 2. Light rays 3 are refracted again at second side wall 6' and strike an optical swamp 8, which prevents light rays 3 from being reflected back onto vessel 2. The beam guidance of light rays 3 is selected such that on the outside of vessel 2, the light rays extend in horizontal direction and parallel to the top surface of the base for vessel 2.

Despite the fact that light rays 3 approach the boundary surface at the total reflection angle, a small

portion of transmitted light rays 3 penetrates to the inside of vessel 2 and forms an evanescent field inside a boundary layer. This portion decreases exponentially with the distance to boundary surface 7. In this case, the penetration depth of the evanescent field is deeper than the surface roughness of the surface for vessel 2.

Among other things, vessel 2 contains luminophores. The luminophores in the exemplary embodiments shown are optically excited in the boundary layer and emit fluorescent rays 9. The portion of fluorescent rays 9 that penetrates the base of vessel 2 strikes a receiver 10, which is arranged at a distance underneath vessel 2. Receiver 10 is formed either by a PIN detector, a photo-multiplier or an avalanche diode.

In order to improve the detection sensitivity, a polarization filter 11, a receiving optic 12 in the form of a collective lens, as well as an interference filter 13 are installed in front of receiver 10.

Receiver 10 and transmitter 4 are connected to an evaluation unit (not shown here) which may comprise, for example, a microcontroller or the like. The receiving signals present at the output of receiver 10 are evaluated

in the evaluation unit. In addition, the evaluation unit controls transmitter 4.

Vessel 2 contains an assay medium, which is typically a watery solution. The assay medium contains a first reaction agent that is detected quantitatively by device 1 according to the invention. First reaction agents typically are antigens or antibodies. A second reaction agent, for example a protein, is bonded by means of adsorption to boundary surface 7 of vessel 2.

The first reaction agent in solution is labeled with the luminophore. The second reaction agent is bonded to the boundary surface 7 of vessel 2. If the luminophore-labeled first reaction agent from the solution bonds to the second reaction agent at the boundary surface, it can then be excited by the evanescent field of the totally reflected transmitted light rays 3 and can emit fluorescent rays 9. These fluorescent rays 9 are quantitatively detected in receiver 10 and are directly proportional to the bonded, luminophore-labeled reaction agent and thus directly proportional to the amount of the originally existing first reaction agent in the solution.

Since only the luminophore bonded to the surface is positioned in the evanescent field of the transmitted light

rays 3, only this luminophore is excited and emits fluorescent rays 9. Non-bonded luminophore in the solution is not positioned in the evanescent field, is therefore not excited and thus does not emit any fluorescent rays 9. This arrangement consequently permits the quantitative determination of bonded luminophore in the presence of non-bonded luminophore, without requiring a prior separation and washing step.

After adding the first reaction agent to vessel 2, it makes sense in this case to directly measure the increase in the bonded luminophore over time as the reaction progresses. Since the amount of bonded luminophore is directly proportional to the originally existing amount of luminophore, the sensor arrangement makes it possible to make a quantitative determination of reactant in the solution, in real time and without additional washing and/or pipetting steps, except for the initial pipetting step.

Since the absorption coefficients and the emission properties for luminophores are very favorable, there are low detection limits. Reactions can be measured and quantified after several minutes already.

When evaluating the receiving signals present at the receiver 10, the dark noise of receiver 10, and the photo counting unit integrated therein, is initially emitted prior to analyzing the immunoassay. In the process, the square root $\sqrt{N}$ of the recorded rate N for the photons is determined as the standard deviation for the dark noise.

For a quantitative detection of the first reaction agent in vessel 2, the receiving signal that increases during the analysis of the immunoassay must reach a multiple of the standard deviation during a specified measuring interval. At the same time, the shape of the increase of the receiving signal must satisfy the shape of an exponential function with a time constant within predetermined tolerance limits. The mathematically adapted values for the time constant, the amplitude and the offset of the receiving signal must be within predetermined limits. Finally, the sum of the deviations from measured values and the mathematical curve, adapted to these, must not exceed a specified value.

The aforementioned parameters can vary corresponding to the changing content in vessel 2.

The design for vessel 2 follows from Figure 1 and, in particular, from Figures 2 and 3. Vessel 2 essentially comprises a hollow-cylindrical base body and is open toward the top.

The base 2′ of vessel 2 consists of a massive circularly cylindrical base body, wherein the envelope surface is slanted on opposite sides. The resulting sidewalls 6, 6′ approach the flat underside 6′′of the base at a slanted angle. The flat sidewalls 6, 6′ are arranged in mirror symmetry to base 2′ body of vessel 2. The inclination angles of the surfaces of sidewalls 6, 6′ for vessel 2 are adapted so that, after accounting for the refraction indices of the vessel material and the assay medium contained inside vessel 2, light rays 3 will be refracted at side wall 6 of vessel 2 so as to strike boundary layer 7 at the total reflection angle as shown in Figure 1. After being reflected, light rays 3 are then correspondingly coupled out via second sidewall 6′ of vessel 2.

Referring to Figures 2 and 3, vessel 2 has an attachment 14, which adjoins the upper edge of the hollow cylindrical base body. Attachment 14 is shaped like a disk and has essentially the shape of a plate with rectangular

cross section. Vessel 2 is secured relative to the sensor arrangement by securing it with attachment 14 to a holder that is not shown here. Vessel 2 preferably is secured with opposite-arranged side edges of attachment 14. The side edges or the top surface of attachment 14 can also have markings for identifying the content of vessel 2. In particular, these markings can be in the form of bar codes.

Vessel 2, comprising a base body and attachment 14, is preferably formed as one piece and more preferably consists of an extrusion-molded plastic part. Vessel 2 in this case preferably is made of polystyrene.

The open top of vessel 2 can be closed off with a foil or the like. Following that, the immunoassay to be analyzed can be injected through the foil into vessel 2, for example with an injection needle.

With the arrangement according to Figure 1, light rays 3 that are emitted by transmitter 4 are coupled into the vessel 2 and, following a one-time total reflection at the boundary surface 7 of vessel 2, are again coupled out of the vessel and finally transmitted to optical swamp 8. The disadvantage here is that only a small portion of the luminophores present in the boundary layer is optically excited, owing to the small diameter of the transmitted

light rays 3, which typically is much smaller than the size of boundary layer 7.

The arrangement according to Figure 4 can be provided to remedy this disadvantage. Following the first passage through vessel 2, this arrangement permits light rays 3 to be reflected on a first mirror 15 with upstream installed polarization filter 16 and to be coupled into vessel 2 once more by second side wall 6' of base 2', such that transmitted light rays 3 are again reflected with the total reflection angle on boundary surface 7. Transmitted light rays 3, which exit from vessel 2 following the reflection, subsequently strike a second mirror 17 with upstream-installed polarization filter 18 and are again coupled into vessel 2. Following a total reflection at boundary surface 7, light rays 3 again strike the mirror 15. From there, light rays 3 are once more coupled into vessel 2 and, following total reflection at boundary surface 7, strike optical swamp 8. Transmitter 4, mirrors 15, 17, as well as optical swamp 8, are essentially arranged in one plane that extends through the vessel base and are displaced to the side, relative to each other, so that during the individual passages through vessel 2, light rays 3 arrive at respectively different locations of boundary surface 7. As

a result, a considerable portion of the boundary surface 7 is optically excited by means of transmitted light rays 3.

Figures 5 and 6 show a device 100 with an arrangement of multiple vessels 2. Vessels 2 in this case are arranged in a line, one after another, and are positioned inside a frame 19, which serves as a holder. A transmitter 4 and an optical swamp 8 are respectively assigned to each vessel 2.

Fluorescent rays 9, exiting on the underside of the bases for the vessels 2, are conducted via receiving optic 12 to receiver 10. An interference filter and a polarization filter are installed in front of receiver 10, in the same way as in Figure 1. These are not shown in Figures 5 and 6 for reasons of clarity.

A lens 20 and a subsequently installed reflecting mirror 21 are arranged below each vessel 2. Transmitted light rays 3 that exit from vessel 2 are collected by means of lens 20 and are focused onto reflecting mirror 21. The respective reflecting mirrors 21, which are assigned to individual vessels 2, are oriented such that the thereon-reflected fluorescent rays 9 arrive at receiver 10 by way of receiving optic 12. For reasons of clarity, only fluorescent rays 9 that emanate from three vessels 2 are

shown in Figure 5. The fluorescent rays 9 emanating from the remaining vessels 2 are not drawn in.

With the aid of an evaluation unit (not shown), transmitters 4 are individually activated one after another, so that fluorescent rays 9 exiting from separate vessels 2 can be evaluated separately.

Figures 7 and 8 show another embodiment according to the invention with a different arrangement of multiple vessels 2. Figure 7 shows a concentric arrangement of vessels 2, wherein a stationary polygonal mirror 22 is arranged in the center of the arrangement. Each vessel 2 in turn is coordinated with a transmitter 4 and an optical swamp 8, which are not shown in Figures 7 and 8. Vessels 2 are positioned in a non-depicted holder. Transmitters 4 and optical swamps 8 are also arranged stationary in the specified positions relative to the respective vessel 2.

As can be seen especially in Figure 8, vessels 2 are positioned in one plane, while polygonal mirror 22 is located underneath vessels 2. A lens 23 and a reflecting mirror 24 are assigned to each vessel 2 and are arranged below the base of the respective vessel 2. Fluorescent rays 9 that exit from the bottom of each base for the respective vessel 2 are collected with lens 23 and focused onto

reflecting mirror 24. The thereon-reflected fluorescent rays 9 are then deflected toward polygonal mirror 22 and are conducted to receiver 10, arranged underneath polygonal mirror 22. A receiving optic, which is installed in front of receiver 10, as well as a polarization filter and an interference filter, although not shown in Figure 8, are employed in a manner similar to that shown and discussed in connection with Figure 1.

Transmitters 4 in turn are activated individually, one after another, so that fluorescent rays 9 that exit from individual vessels 2 can be evaluated separately.

The illustrated exemplary embodiments of the device according to the invention can also be expanded to use luminophores that emit fluorescent rays 9 and/or phosphorescent rays. In particular, organic and organometallic coloring agents are used in this case.

Since the phosphorescent radiation has a longer emission interval the fluorescent radiation, the phosphorescent radiation is emitted with a delay relative to the fluorescent radiation, wherein the delay times are in the µs to ms range.

In the simplest case, vessel 2 contains only one type of first reaction agents, which are labeled with a luminophore. Depending on the luminophore composition, these emit phosphorescent rays and/or fluorescent rays 9, which are then evaluated in receiver 10.

Desirably, the detection of the phosphorescent rays occurs by means of a photon count in receiver 10, while transmitter 4 is turned off.

In one advantageous embodiment, vessel 2 of the invention contains two different types of first reaction agents, which are labeled with different luminophores. The first luminophores have a high fluorescence and low phosphorescence, so that they essentially emit only fluorescent rays 9 when optically excited. The second types of luminophores have high phosphorescence and a low fluorescence, so that they essentially emit only phosphorescent rays when optically excited.

In order to be able to detect the phosphorescent radiation separately from the fluorescent rays 9, transmitter 4 is operated in the pulsed mode. The pulse-break ratio of the light pulses, emitted by transmitter 4, is selected such that fluorescent rays 9 are emitted during

the emission of light pulses and phosphorescent rays are emitted during the transmitting breaks.

In this way, both types of first reaction agents can be detected quantitatively through a separate analysis of the fluorescent rays of the first luminophores, tied to the first type of reaction agent, and the phosphorescent rays of the second luminophores, tied to the second type of the first reaction agent.

It will be understood that the above description of the present invention is susceptible to various modifications, changes and adaptations, and the same are intended to be comprehended within the meaning and range of equivalents of the appended claims.